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PCR bias in amplification of androgen receptor alleles, a trinucleotide repeat marker used in clonality studies.

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Trinucleotide CAG repeats in the X-linked human androgen receptor gene (HUMARA) have proved a useful means of determining X chromosome haplotypes, and when combined with methylation analysis of nearby cytosine residues permits identification of non-random X inactivation in tumors of women. Co-amplification of two alleles in a heterozygote generates PCR products which differ in the number of CAG units, and thus their melting and secondary structure characteristics. We have shown that under optimal conditions amplification efficiency of two HUMARA alleles is near-equivalent, generating PCR products in a ratio proportional to that of the genomic template. In contrast, reduction of template quantity, damage of template by ultraviolet irradiation or addition of monovalent salts (sodium chloride, sodium acetate or ammonium acetate) produces highly variable imbalances of allelic PCR products, with a strong tendency to preferentially amplify lower molecular weight alleles. Variability and biasing was diminished by substitution of 7-deaza-2'-dGTP for dGTP during amplification, an intervention which reduces stability of intramolecular and intermolecular GC base pairing. We conclude that DNA which is scanty, damaged or salt contaminated may display amplification bias of GC-rich PCR targets, potentially confounding accurate interpretation or reproducibility of assays which require co-amplification of alleles.

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